contacting the loaded support means with the liquid sample to be analyzed, such that each of the spots is contacted in the same step with said liquid sample, the amount of liquid used in said sample being such that only an insignificant proportion of any analyte present in said liquid sample becomes bound to said binding agent specific for said analyte, and

measuring a parameter representative of the fractional occupancy by said analytes of said binding agents at the spots by a competitive or non-competitive assay technique using a site-recognition reagent for each binding agent capable of recognizing either the unfilled binding sites or the filled binding sites on said binding agent, said site-recognition reagent being labelled with a marker enabling the amount of said reagent in the particular location to be measured.

B1

2 ~~13.~~ A method as claimed in claim 1 ~~12,~~ wherein each of said spots has a size of less than 1 mm^2.

3 ~~14.~~ A method as claimed in claim 2 ~~13,~~ wherein each of said spots contains more than 10^4 molecules of binding agent.

4 ~~15.~~ A method as claimed in claim 3 ~~14,~~ wherein each of said spots has less than 0.01 V/K moles of binding agent.

5 ~~16.~~ A method as claimed in claim 3 ~~14,~~ wherein said C binding agents used have affinity ~~equilibrium~~ constants for said analytes of from 10^8 to 10^{13} liters per mole.

6 ~~17.~~ A method as claimed in claim 3 ~~14,~~ wherein said C binding agents used have affinity ~~equilibrium~~ constants for said analytes of the order of 10^{10} or 10^{11} liters per mole.

7 ~~18.~~ A method as claimed in claim 3 ~~14,~~ wherein the volume of said liquid sample is not more than 0.1 liter.

8 ~~19~~. A method as claimed in claim 3 ~~14~~, wherein the volume of said liquid sample is 400 to 1000 microliters.

9 ~~20~~. A method as claimed in claim 1 ~~12~~, wherein said binding agents loaded onto said support means are antibodies for the analytes whose concentrations are to be determined.

10 ~~21~~. A method as claimed in claim 1 ~~12~~, wherein said binding agents are labelled with markers enabling the concentration levels of said binding agents to be measured.

B' 11 ~~22~~. A method as claimed in claim 10 ~~21~~, wherein said binding agents and said site-recognition reagents are labelled with fluorescent markers such that at the individual spots the assay technique for measuring fractional occupancy of the binding agents measures the ratios of the signals emitted by the fluorescent markers.

12 ~~23~~. A device for use in determining the ambient concentrations of a plurality of analytes in a liquid sample of volume V liters, comprising a solid support means having located thereon at high coating density at a plurality of spaced apart small spots a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in said liquid sample and is specific for said analyte as compared to the other components of said liquid sample, each spot having not more than 0.1 V/K moles of a single binding agent, where K liters/mole is the affinity ~~equilibrium~~ constant of said single binding agent for reaction with the analyte to which it is specific.

C

13 ~~24~~. A device as claimed in claim 12 ~~23~~, wherein each of said spots has a size of less than 1 mm^2.

14 ~~25~~. A device as claimed in claim 13 ~~24~~, wherein each of said spots contains more than 10^4 molecules of binding agent.

15
~~26~~. A kit for use in determining the ambient concentration of a plurality of analytes in a liquid sample of volume V litres, comprising:

a solid support means having located thereon at high coating density at a plurality of spaced apart small spots a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in said liquid sample and is specific for said analyte as compared to the other components of the liquid sample, each spot having not more than 0.1 V/K moles of a single binding agent, where K liters/mole is the affinity ~~equilibrium~~ constant of said single binding agent for reaction with the analyte to which it is specific;

a plurality of standard samples containing known concentrations of the analytes whose concentrations in the liquid sample are to be measured; and

a set of labelled site-recognition reagents for reaction with filled or unfilled binding sites on said binding agents.

16
~~27~~. A kit as claimed in claim 15 ~~26~~, wherein each of said spots has a size of less than 1 mm^2.

17
~~28~~. A kit as claimed in claim 16 ~~27~~, wherein each of said spots contains more than 10^4 molecules of binding agent.

Cancel claims 1-11.

Newly added claims 12-28 are fairly based on the disclosure of published International Application No. PCT/GB88/00649 (WO 89/01157) on which the present application is based. See specification at page 14, line 24 through page 5, line 2 and at page 12, lines 2-5.

As reflected in the claims submitted herewith, the invention on which applicant seeks protection is a method for determining ambient concentrations of a plurality of analytes involving, in combination, the use of a small amount of

binding agent on a small spot at high coating density. The combination of these three (3) features imparts surprisingly high sensitivity to the method of the invention. The use of a small amount of binding agent, as proposed in WO 84/01031, which is cited in the International Search Report in the aforesaid WO 89/01157, does not by itself ensure high sensitivity, but rather obviates the requirement of a constant volume sample. Unless a small spot is used, high sensitivity of measurement cannot be achieved because it is impossible to obtain a high signal/noise ratio. The use of a high coating density also contributes substantially to maximizing the signal/noise ratio.

The conventional view before applicant made the present invention was that very small amounts of antibody would pull out only very small amounts of analyte and hence that the signal would be very low and thus the signal/noise ratio would likewise be low. The present invention embodies applicant's surprising discovery, in contravention of the above-noted conventional view, that extremely high sensitivity can be obtained by using a small spot and high coating density.

Favorable consideration and allowance of the claims presented herewith is earnestly solicited.

Respectfully submitted,

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